

Saltmarsh plant responses to eutrophication

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Abstract. In saltmarsh plant communities, bottom-up pressure from nutrient enrichment is predicted to increase productivity, alter community structure, decrease biodiversity, and alter ecosystem functioning. Previous work supporting these predictions has been based largely on short-term, plot-level (e.g., 1–300 m²) studies, which may miss landscape-level phenomena that drive ecosystem-level responses. We implemented an ecosystem-scale, nine-year nutrient experiment to examine how saltmarsh plants respond to simulated conditions of coastal eutrophication. Our study differed from previous saltmarsh enrichment studies in that we applied realistic concentrations of nitrate (70–100 µM NO₃[−]), the most common form of coastal nutrient enrichment, via tidal water at the ecosystem scale (~60,000 m² creeksheds). Our enrichments added a total of 1,700 kg N·creek^{−1}·yr^{−1}, which increased N loading 10-fold vs. reference creeks (low-marsh, 171 g N·m^{−2}·yr^{−1}; high-marsh, 19 g N·m^{−2}·yr^{−1}). Nutrients increased the shoot mass and height of low marsh, tall *Spartina alterniflora*; however, declines in stem density resulted in no consistent increase in aboveground biomass. High-marsh plants *S. patens* and stunted *S. alterniflora* did not respond consistently to enrichment. Nutrient enrichment did not shift community structure, contrary to the prediction of nutrient-driven dominance of *S. alterniflora* and *Distichlis spicata* over *S. patens*. Our mild responses may differ from the results of previous studies for a number of reasons. First, the limited response of the high marsh may be explained by loading rates orders of magnitude lower than previous work. Low loading rates in the high marsh reflect infrequent inundation, arguing that inundation patterns must be considered when predicting responses to estuarine eutrophication. Additionally, we applied nitrate instead of the typically used ammonium, which is energetically favored over nitrate for plant uptake. Thus, the form of nitrogen enrichment used, not just N-load, may be important in predicting plant responses. Overall, our results suggest that when coastal eutrophication is dominated by nitrate and delivered via flooding tidal water, aboveground saltmarsh plant responses may be limited despite moderate-to-high water-column N concentrations. Furthermore, we argue that the methodological limitations of nutrient studies must be considered when using results to inform management decisions about wetlands.

Key words: coastal wetland; eutrophication; global change; nutrient pollution; plants; salt marsh; *Spartina*.

INTRODUCTION

Human activities have more than doubled the amount of reactive nitrogen (N) in the biosphere (Vitousek et al. 1997, Galloway et al. 2008). As a result, eutrophication (i.e., nutrient enrichment) has become a global issue for a range of ecosystems (e.g., forests, grasslands, oceans, and estuaries). Coastal eutrophication is often driven by watershed activities, such as agricultural intensification and rising human population density (Valiela et al. 1992, Deegan et al. 2012), and heavily impacts salt marshes (Pardo et al. 2011, Deegan et al. 2012). Bottom-up (resource) theory predicts that resource enhancements such as nutrient enrichment should increase plant production. Plant responses to nutrient enrichment, however, are variable and may be mediated by the magnitude of enrichment, soil condition, properties of specific

nutrients, plant traits, life history, and interspecific interactions (Chapin et al. 1986, Isbell et al. 2013, He and Silliman 2015). At the community level, recent theory predicts that eutrophication decreases biodiversity (Bertness and Pennings 2000, Stevens et al. 2004, Hautier et al. 2009, Isbell et al. 2013). Given that plants are frequently foundation species within ecosystems, their response to eutrophication can likely drive changes in both structure and function at the ecosystem-level (Deegan et al. 2012, Isbell et al. 2013). Thus, for any given ecosystem, predicting the responses to nutrient enrichment relies on understanding factors which may influence plant responses.

Based on a long history of plot-level (typically 1–300 m²) fertilization studies, salt marshes are thought to follow the predictions of ecological theory at both population and community levels. Nutrient enrichment generally stimulates aboveground height and mass of individual shoots, which may ultimately increase total aboveground production (dry mass m²/yr; Valiela and Teal 1974, Mendelssohn 1979, Valiela 1984, Darby and

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Turner 2008). At the community level, the literature argues that salt marshes generally follow the same patterns of nutrient-driven reductions in diversity as seen in terrestrial ecosystems (Bertness and Pennings 2000, Isbell et al. 2013). Nutrient enrichment can change marsh plant community structure by altering competitive dynamics (Levine et al. 1998, Bertness et al. 2002, Pennings et al. 2005). For instance, increased N availability can alter competitive hierarchies in saltmarsh plant communities in U.S. marshes resulting in the landward (upslope) encroachment of *S. alterniflora*, the subsequent displacement of *S. patens* (Levine et al. 1998) and high marsh expansion of *Distichlis spicata* (Pennings et al. 2005, Fox et al. 2012).

Previous saltmarsh fertilization studies have been critical in developing conceptual models to understand marsh responses to eutrophication and in turn inform management strategies. Previous studies, however, may not have adequately represented real-world eutrophication. Typical salt marsh fertilization studies use dry, ammonium-based fertilizers (commonly NH_4Cl , urea, NH_4NO_3 , or commercial fertilizers that contain a combination of these) sprinkled on the soil surface or via slow-release pellets at the rooting zone (Valiela and Teal 1974, Levine et al. 1998, Crain 2007, Fox et al. 2012). Nitrate, however, not ammonium, is the dominant N species in eutrophic coastal waters (Cloern 2001), and tidal water is the primary vector for N delivery to coastal marshes. Thus, if mode of delivery (dissolved in water and tidally delivered vs. dry surface or slow-release application) and type of N (NO_3^- vs. NH_4^+) are important in determining ecosystem responses to nutrient enrichment (Mendelsohn 1979), prior experiments may be insufficient to predict eutrophication effects. Furthermore, the small size of these plot-level studies may miss landscape-level phenomena that drive ecosystem-level responses. For instance, the inundation gradient of salt marshes can drive phenotypic differences within a species and result in differential responses between plant phenotypes (Silliman and Bertness 2002). Given that tidal flooding is the most common vector for nutrient delivery to salt marshes, an inundation gradient will also generate a gradient of N loading with highest loading rates in the regularly flooded low marsh and lower loading rates in the irregularly flooded high elevation marsh (Deegan et al. 2007).

We examine vegetation responses in a New England salt marsh exposed to nine years of nitrate enrichment applied at the ecosystem scale. Our experiment is novel in at least four ways. First, nitrogen was added as nitrate (NO_3^-), a mobile form that dominates land-derived N contributing to coastal eutrophication (Cloern 2001). Second, dissolved nutrients were added directly to flooding tidal water to mimic the most common mode of nutrient delivery to tidal marshes. Third, enrichment was manipulated at the ecosystem-level with experimental marsh units representing $\sim 60,000 \text{ m}^2$ of marsh area. Finally, our target enrichment concentrations were ecologically and environmentally realistic, corresponding to

what is defined as moderate to highly eutrophic for coastal systems (USEPA 2005). Following the predictions of bottom-up control of saltmarsh plants, we hypothesized that enrichment would (1) increase individual shoot and total aboveground biomass for each species and (2) alter high-marsh community structure as *S. alterniflora* and *D. spicata* increase, outcompeting *S. patens*.

METHODS

Site description

This study was conducted during nine growing seasons (May–September 2004–2012) in four first-order tidal creeks ($\sim 300 \text{ m}$ long, 15 m wide at the mouth tapering to 2 m near the terminus) in the Plum Island Sound Estuary, Massachusetts, USA. Mean tide range at the mouth of the estuary is 2.63 m . These creeks are located within the large expanse of salt marsh (40 km^2) within the Rowley River sub-estuary ($42^\circ 44' \text{ N}$, $70^\circ 52' \text{ W}$). Creeks were similar in length ($300\text{--}500 \text{ m}$), volume ($4.1\text{--}7.5 \times 10^6 \text{ L}$; Johnson et al. 2007, Deegan et al. 2012), and physico-chemical characteristics (Deegan et al. 2007).

The experimental marshes are typical New England saltmarsh communities with an extensive high marsh (i.e., elevations above mean high water) dominated by *S. patens*, *D. spicata*, and stunted *S. alterniflora*, with tall *S. alterniflora* along creek banks and bay fronts (Miller and Egler 1950, Niering and Warren 1980, Bertness 1991; Fig. 1; see Appendix S1: Table S1 for species percent cover and frequency of occurrence). Creek-bank, tall *S. alterniflora* occurs in a $2\text{--}3 \text{ m}$ wide band along the marsh edge, while stunted *S. alterniflora* exists in discrete patches on the high marsh. The upland marsh edge is dominated by *Iva frutescens* interspersed with small patches of *Phragmites australis*.

Experimental design

Nutrient enrichment.—Two primary creeks were selected to receive nutrient enrichment and two primary creeks remained as unenriched references. Prior to experimental enrichment, plant trait and community characteristics were similar among tidal creeks (Deegan et al. 2007). We added nutrients by pumping a concentrated solution of NO_3^- and PO_4^{3-} to the headwaters of the incoming tide of every tide from mid-May to mid-September. Nutrients were added 100 m seaward of experimental plots to allow in-channel mixing to occur before reaching plots. The pump rate was adjusted every 10 min , proportional to the flux of flooding water as a function of average predicted tide height for the day and measured water level. Pumping stopped when the high marsh was flooded by $\sim 20 \text{ cm}$ of enriched water.

Our NO_3^- enrichment target concentration for the water column flooding the marsh was $70\text{--}100 \mu\text{M NO}_3^-$ (added as NaNO_3). This concentration was $10\text{--}15$ times

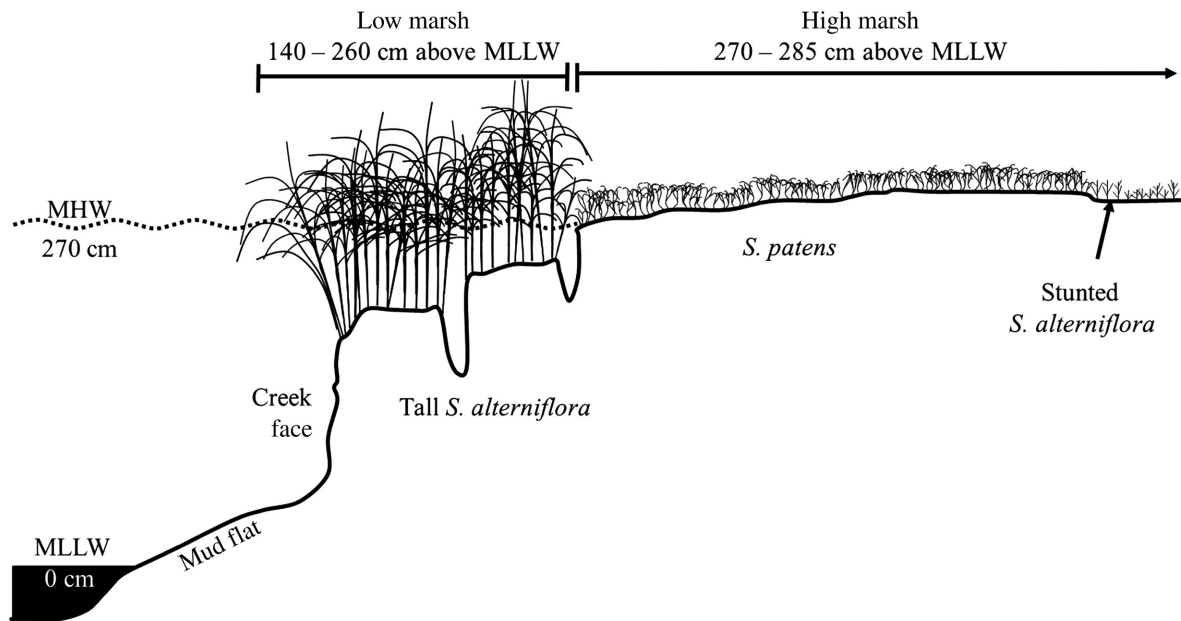


FIG. 1. Cross-section of a tidal creek at low tide illustrating saltmarsh vegetation patterns in the Plum Island Estuary, Massachusetts, USA. MLLW, mean low-low water; MHW, mean high water.

over Plum Island water column background ($5\text{--}10\ \mu\text{M}\ \text{NO}_3^-$; Table 1). The change in Na concentration was negligible (0.01% ; $0.0016\ \text{g Na/L}$ added to $11\ \text{g N/L}$ present in seawater). Initially we added PO_4^{3-} (added as NaH_2PO_4) at a target of $5\text{--}7\ \mu\text{M}$ to achieve an approximately Redfield ratio ($15:1\ \text{N:P}$) in added nutrients to avoid secondary P limitation. Our analysis (colorimetric method; Parsons et al. 1984) found that creek water P was in excess, thus after 2010 we discontinued the P addition. Final in-creek water-column concentrations varied from the targets (Table 1) because actual water flux varied with real vs. predicted tide height. From 2004 to 2010 (years of enrichment 1–7), nutrients were added for at least 120 d. To emphasize the scale of our additions, we added an average of 12 tons of NaNO_3 ($17 \times 10^5\ \text{g N as NO}_3^-$) commercial fertilizer to each creek replicate in these

years. Due to global nitrate fertilizer shortages in 2011 (year 8), creeks were enriched at half concentration ($35\ \mu\text{M}\ \text{NO}_3^-$) for the first 55 d, no nutrients were added for the next 20 d, and we were able to return to our target concentration ($70\text{--}100\ \mu\text{M}\ \text{NO}_3^-$) for the remaining 45 d of the growing season. Again, due to fertilizer shortages in 2012 (year 9), nitrate was added at full concentration starting in mid-June (instead of mid-May) until mid-July, when it was lowered to half concentration for 20 d then raised full concentration for 14 d and then ended at the beginning of September.

Elevation and hydroperiod.—To evaluate the relative elevation between reference and enriched creeks and to determine flooding heights and frequency, fine scale elevation profiles were determined across transects at three

TABLE 1. Mean ($\pm 1\ \text{SE}$; $n = 10\text{--}36$) flooding water-column nitrate concentrations ($\mu\text{mol/L}$).

Year	Reference	Enriched	Data source
2003–pre-treatment	3.5 ± 0.4	4.3 ± 0.3	Deegan et al. (2007)
2004	4.2 ± 0.3	71 ± 38	this study, Deegan et al. (2007)
2005	6.9 ± 0.5	95 ± 18	this study, Deegan et al. (2007)
2006	7 ± 1	105 ± 12	this study, Koop-Jakobsen and Giblin (2010)
2007	7 ± 2	132 ± 11	this study, Koop-Jakobsen and Giblin (2010)
2008	4.2 ± 0.7	79 ± 10.8	this study
2009	2.9 ± 1.0	61 ± 9	this study
2010	6.7 ± 0.3	123 ± 20	this study, Vieillard and Fulweiler (2012)
2011	2.7 ± 0.1	69 ± 12	this study, Vieillard and Fulweiler (2012)
2012	21 ± 3	118 ± 9	this study

Notes: Data from unpublished and published sources. For this study, Deegan et al. 2007 and Koop-Jakobsen and Giblin 2010, nitrate analysis as described in *Methods*. Vieillard and Fulweiler (2012) analyzed samples on a SEAL Autoanalyzer 3 instead of a Lachat Quik Chem 8000.

points along each creek. These transects were at right angles to the creek channel axis and extended landward 50 m from the break between tall *S. alterniflora* and high marsh *S. patens*. The break was designated as "0 m." Temporary benchmarks were established for each reference and enriched creek in late June 2005 by pounding 150-cm long oak stakes into the peat, leaving ~15 cm exposed above the marsh surface. The elevation at the top of each stake relative to mean lower low water was determined using "tide sticks" (Smith and Warren 2007) deployed beside each temporary benchmark. Surface elevation relative to these temporary benchmarks was measured at 1-m intervals along each transect using a Topcon GTP 2003 Total Station, Itabashi-ku, Tokyo, Japan. Based on these measurements, mean high marsh and low marsh elevations were calculated for each transect. Tide heights were calculated using the NOAA correction as 0.94 times the recorded tide height at the NOAA Portland, Maine, USA, tide gauge (Station ID: 8418150,) using the mean of three spring tides in June, July, and August 2005.⁶ Elevations were converted to growing season (May–September) hydroperiods, tidal flooding frequency and duration, using tidal height in the program Tide Miner.⁷

Nutrient concentrations and loading.—Nitrate concentrations were measured at least once a summer via autosamplers (SIGMA, Loveland, Colorado, USA) taking hourly samples for 24 h. These samples were taken from the water column ~0.25 m off the creek bottom. Data reported are based on samples taken during mid-flood tides. Synoptic sampling was supplemented by twice-monthly water samples collected by hand 0.25 m beneath the surface of the creek water during mid-flood tide. Water samples were collected in acid washed bottles. Samples were refrigerated and filtered (pre-combusted GFF filter) within 24 h of collection and frozen until analysis, and NO_3^- concentration was determined colorimetrically (Wood et al. 1967; Lachat Quik Chem 8000, Lachat Zellweger Instruments, Milwaukee, Wisconsin, USA).

We found no consistent difference in nutrient concentration in the water column with depth or between the creek and the marsh surface at high tide, probably because of the rapid flooding rate in this system (Deegan et al. 2007). We defined nutrient loading to be the amount of nutrient present in the water over a m^2 of marsh at high tide, as this is the most equivalent to estimates of nutrient loading by dry fertilizer additions. Thus, nutrient loading to a particular habitat, or marsh elevation, is a function of water column nutrient concentration and the maximum depth of flooding for a tide. Loading rates were calculated for three habitats along an inundation gradient: the creek bottom (unvegetated, muddy benthos), low marsh (tall *Spartina alterniflora*), and high marsh (*S. patens*, stunted *S. alterniflora*). Daily loading rates by habitat were calculated for each tide by estimating the water

depth from a tide table and marsh elevation multiplying by mean water-column nitrate concentration. Annual loading ($\text{g N}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$) was estimated by summing daily loading rates over all the tides when the marsh was being enriched.

Plant responses

Shoot height, mass, and shoot-specific mass.—Vegetation was sampled along the same transect used to determine marsh elevation profiles. Transects intersected the dominant vegetation types but did not extend to the *Iva*-dominated upland marsh edge. Vegetation patterns along these transects prior to nutrient enrichment were similar between enriched and reference creeks (Deegan et al. 2007). Aboveground biomass (shoot dry mass), shoot length, and shoot-specific mass ($\text{g stem}/\text{cm}$) were measured for 25 individual shoots of tall *S. alterniflora*, *S. patens*, stunted *S. alterniflora*, and *D. spicata*, located haphazardly within 4 m of each transect. All plants were cut at the marsh surface, refrigerated within 2 h of collection, washed individually in the lab to remove sediments, measured for length, dried at 80°C to a constant mass, and weighed. Measurements were taken bi-weekly in 2005 and at the end of the season all other years (mid-August 2004–2012). Tall *S. alterniflora*, *S. patens*, and stunted *S. alterniflora* were sampled throughout the study period. *D. spicata* shoot measurements began in 2006.

Foliar and standing stock N.—Foliar percent nitrogen by mass was determined from two to three pooled leaves for *S. alterniflora*, whereas whole shoots were used for *S. patens* and *D. spicata*. Tall *S. alterniflora*, *S. patens*, and stunted *S. alterniflora* were sampled in all years (2004–2012). *D. spicata* was measured in 2004–2010, except 2005. Analyses were conducted using a PerkinElmer 2400 Series II CHNS/O analyzer (PerkinElmer Life and Analytical Sciences, Wellesley, Massachusetts, USA) at the Marine Biological Laboratory, Massachusetts.

End-of-season aboveground live standing crop and standing stock N.—Stem density (shoots per unit area) was sampled in mid-to-late August with four haphazardly selected areas within 5 m of each transect line within tall *S. alterniflora* (2004–2010), stunted *S. alterniflora* (2003–2005), and *S. patens* (2003–2005). Because of differences in densities among species, different sampling-unit areas were used for each: a 0.25 m^2 quadrat for tall *S. alterniflora*, 0.008 m^2 circle for *S. patens*, and a 0.0625 m^2 quadrat for stunted *S. alterniflora*.

We used end-of-season live (EOSL) standing crop as a conservative proxy for aboveground production. Although EOSL is widely used as a proxy for aboveground production, it can underestimate net aboveground production by up to 15% because it does not account for tissue lost due to factors such as herbivory, self-thinning, or leaf senescence (Nixon and Oviatt 1973). Herbivory in current-study marshes, however, is low (Johnson and Jessen 2008) and

⁶ <http://tidesandcurrents.noaa.gov/index.shtml>

⁷ <https://github.com/cwarren/tideminor>

seasonal stem loss through self-thinning is ~10–15% of mid-August EOSL (Chaisson 2012). EOSL was estimated for each transect as stem densities standardized to m^{-2} multiplied by corresponding mean shoot mass as described previously. Aboveground standing stock N (g N/m^2) was estimated for each transect as EOSL multiplied by corresponding mean percent nitrogen.

Plant community composition.—Plant community composition was sampled in July (2004–2009 and 2011) using visual estimates of percent cover in contiguous 1- m^2 plots along each transect and included the lower limit of tall *S. alterniflora*. Percent cover (a mean of all plots per transect) and frequency of occurrence (percentage of plots within a transect in which a species was found) was determined for each species and transect.

Statistical analysis.—All analyses were conducted in R (R Development Team 2012). To detect differences between enriched and reference creeks for aboveground univariate response variables, between-subjects repeated measures analysis was performed using the lme function in the nlme package in R (*available online*).⁸ Treatment (enriched, reference) was the between-subjects factor, year was the within-subjects factor and transects were the subjects. Based on the lowest Akaike's information criterion values, an autoregressive process of one order was selected for the correlation structure. Data were transformed as needed based on results of homogeneity of variance and normality tests.

To detect differences in plant communities between treatments, analysis of similarities (ANOSIM) was conducted on percent cover and frequency of occurrence data. Plant communities were defined and analyzed separately both by average percent cover and frequency of occurrence of dominant species (tall and stunted *S. alterniflora* considered separate species for this analysis) per transect. ANOSIMs were conducted based on a Bray–Curtis dissimilarity matrix using the anosim function in the vegan package in R (*available online*).⁹

To compare communities, a non-metric multidimensional scaling (nMDS) plot was generated in the eighth year of enrichment (2011). nMDS was conducted by analyzing percent cover data using the metaMDS function on a Bray–Curtis dissimilarity matrix in the MASS package in R (*available online*).¹⁰

RESULTS

Elevation and hydroperiod.—Mean elevation between experimental and reference creeks did not differ for the high marsh ($P = 0.26$), transect origin points ($P = 0.06$), and low marsh ($P = 0.14$; Appendix S1: Table S2). The high marsh was flooded by ~30% of the high tides over

the May–September growing seasons and submerged 4% of the time. The low marsh was flooded by almost 100% of high tides and on average was submerged ~31–38% of the time (Appendix S1: Table S2).

Nutrient concentrations and loading.—Mean water-column nitrate concentrations (Table 1) were 6- to 18-fold higher in enriched creeks (61–132 $\mu\text{mol/L}$) vs. reference creeks (3–21 $\mu\text{mol/L}$). Enriched flooding water added ~1,700 kg N to each experimental creek watershed each year, which would provide in a loading rate of 30 $\text{g N}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ if spread uniformly over the watershed. Mean N loading, adjusted by tidal inundation of each habitat, was ~10-fold higher in enriched creeks vs. reference creeks (low marsh, 171 $\text{g N}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ in enriched creeks vs. 16 $\text{g N}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ in reference creeks; high marsh, 19 $\text{g N}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ in enriched creeks vs. 2 $\text{g N}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ in reference creeks; Table 2; Appendix S1: Table S3).

Shoot height, mass, and shoot-specific mass.—On average, tall *S. alterniflora* shoots increased in height with nutrient enrichment ($P < 0.01$; Fig. 2). Similarly, *D. spicata* had greater shoot heights ($P < 0.01$) with nutrient enrichment in years 3–5, but then subsequently reversed with taller shoots in reference creeks in years 6–8. No significant differences were observed in shoot height between treatments in either *S. patens* and stunted *S. alterniflora* (Fig. 2).

Tall *S. alterniflora* shoots increased in mass with enrichment ($P < 0.01$). Stunted *S. alterniflora* shoot mass was also significantly greater with enrichment ($P < 0.01$), but there was an interaction between year of enrichment and nutrient effects ($P = 0.03$). Nutrient enrichment ($P < 0.01$) and year ($P < 0.01$) had significant effects on *D. spicata* shoot mass, with more massive stems in reference creeks for most years. There was no effect of year or enrichment on *S. patens* shoot mass.

Tall *S. alterniflora* shoots increased shoot-specific mass with nutrient enrichment ($P < 0.01$; Fig. 2). Enrichment did not affect *S. patens* shoot-specific mass, but there was significant variation among years ($P = 0.05$; Fig. 2). Nutrient enrichment significantly increased the shoot-specific mass of stunted *S. alterniflora* and *D. spicata* ($P < 0.01$; Fig. 2).

Stem densities and end-of-season live standing crop.—In all creeks, tall *S. alterniflora* stem density exhibited

TABLE 2. N loading rates ($\text{g N}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$; ± 1 SE) by habitat averaged across years ($n = 9$) for enriched and reference creeks based on hydrology and measured water-column nutrient concentrations.

Year	Low-marsh	High-marsh	Creek bottom
Enriched	171 \pm 19	19 \pm 2	686 \pm 75
Reference	16 \pm 4	2 \pm 1	63 \pm 16

⁸ <http://cran.r-project.org/web/packages/nlme/index.html>

⁹ <http://cran.r-project.org/web/packages/vegan/index.html>

¹⁰ <http://cran.r-project.org/web/packages/MASS/index.html>

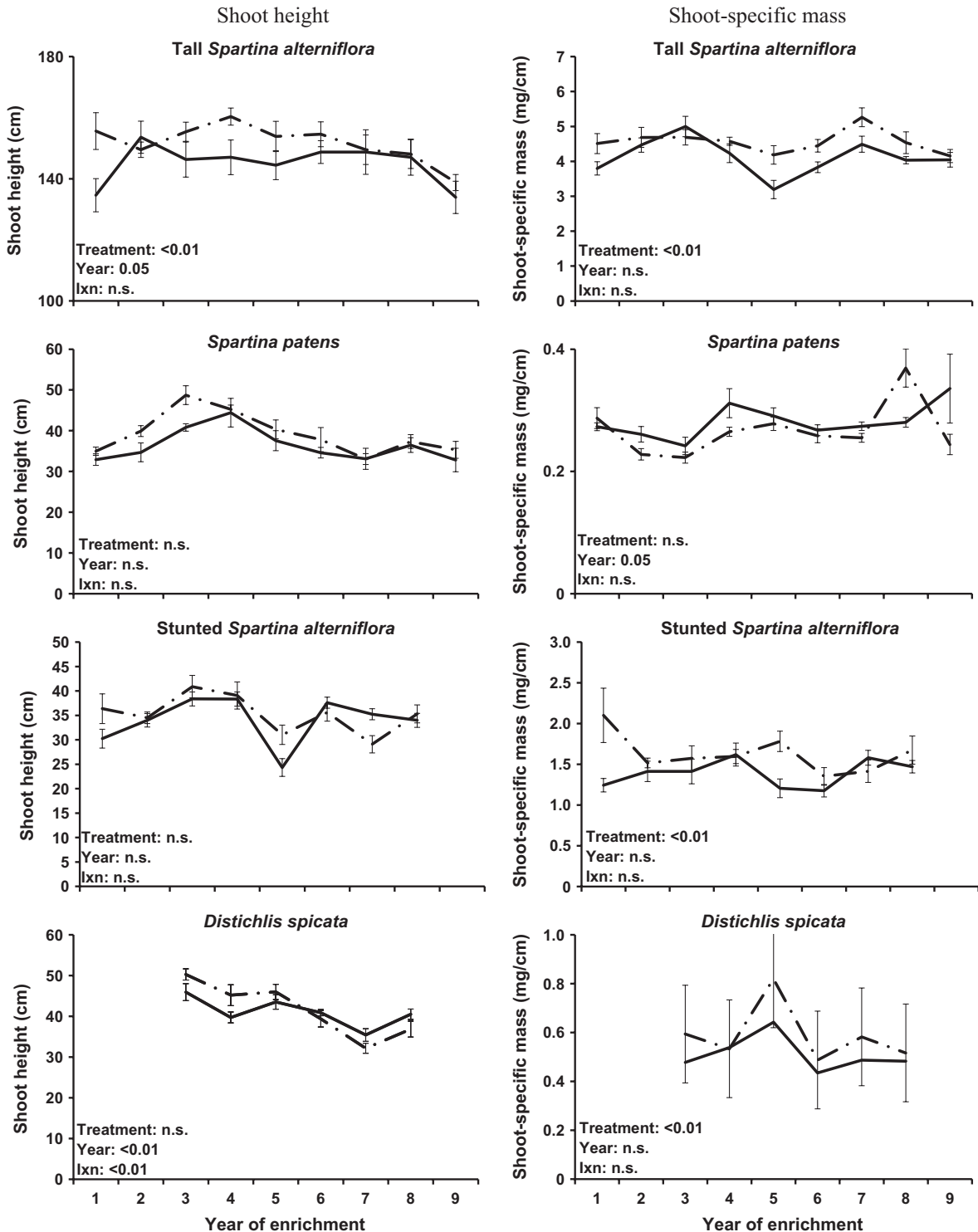


FIG. 2. Mean ($1 \pm \text{SE}$) of shoot height (cm) and shoot-specific mass (mg/cm) for tall and stunted *Spartina alterniflora*, *S. patens*, and *Distichlis spicata* in enriched (dashed line) and reference creeks (solid line). Statistical P values based on repeated-measures ANOVAs for effects of treatment (enrichment vs. reference) and year. Abbreviations are n.s., not significant; Ixn, interaction between year and treatment.

considerable inter-annual variability (Fig. 3; $P = 0.34$), with lower stem densities in the nutrient enriched marshes ($P = 0.06$). *S. patens* stem densities exhibited a striking year effect ($P < 0.01$) with a system-wide increase in year 3 of enrichment, but density did not differ between treatments (Fig. 3). Stunted *S. alterniflora* stem densities were significantly different between treatments ($P = 0.01$), with stem densities frequently lower in enriched creeks than in reference creeks (Fig. 3).

Nutrient enrichment had no overall significant effect on end-of-season live biomass for tall *S. alterniflora*, stunted *S. alterniflora*, *S. patens*, and *D. spicata* (Fig. 3).

We observed significant inter-annual variability with significant increases of ESOL in year 3 vs. year 1 for both *S. patens* and stunted *S. alterniflora* ($P < 0.01$; Fig. 3).

Foliar and standing stock N.—Enrichment increased foliar percent N in stunted *S. alterniflora* ($P < 0.01$) for all years but the magnitude of increase varied by year (Fig. 4; treatment \times year interaction, $P < 0.01$). Enrichment did not affect foliar N in tall *S. alterniflora*, *S. patens*, or *D. spicata* ($P \geq 0.15$). Standing stock N (g N/m^2) for tall *S. alterniflora* had high inter-annual variability (Fig. 4), and no significant effect of nutrient enrichment was

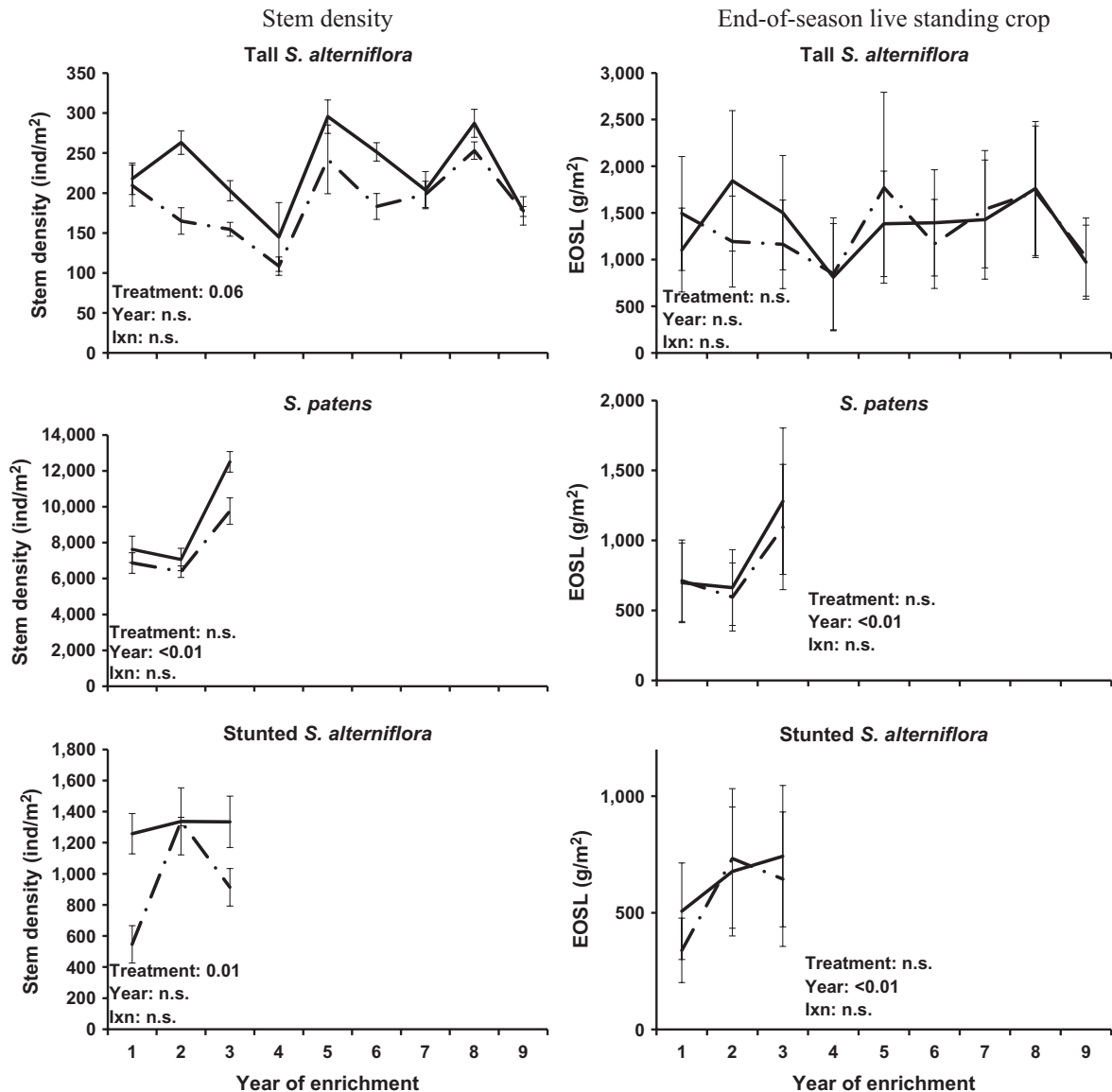


FIG. 3. Mean ($1 \pm \text{SE}$) stem density and end of season live standing crop (EOSL) as a proxy for annual shoot net primary production for tall and stunted *Spartina alterniflora* and *S. patens* in enriched (dashed line) and reference creeks (solid line). Statistical P values based on repeated-measures ANOVAs for effects of treatment (enrichment vs. reference) and year. Abbreviations are n.s., not significant; Ixn, interaction between year and treatment.

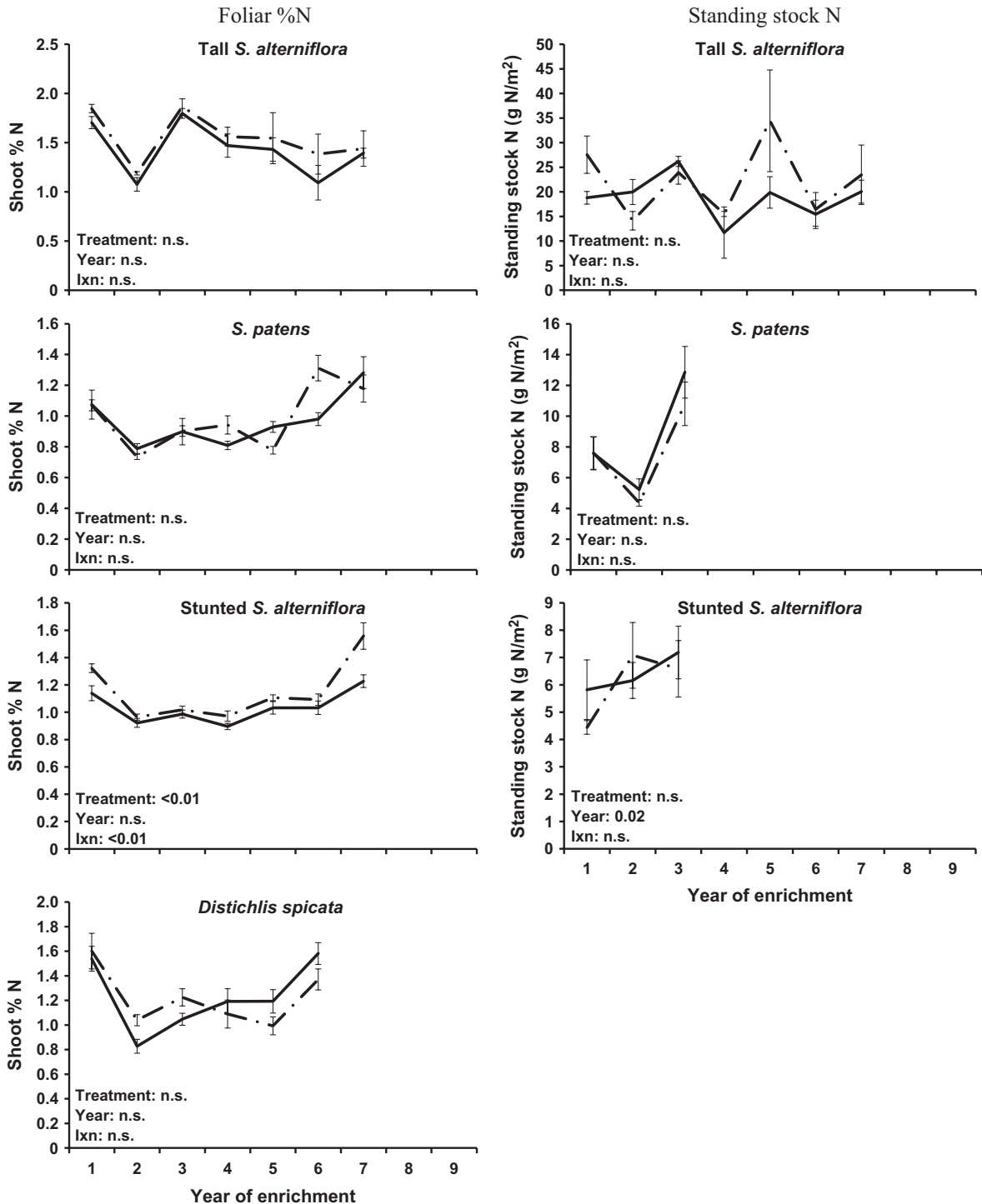


FIG. 4. Mean ($1 \pm \text{SE}$) of foliar percent nitrogen and standing stock N in the leaves of tall and stunted *Spartina alterniflora*, *S. patens*, and *Distichlis spicata* (foliar N only) in enriched (dashed line) and reference creeks (solid line). Statistical *P* values based on repeated-measures ANOVAs for effects of treatment (enrichment vs. reference) and year. Abbreviations are n.s., not significant; Ixn, interaction between year and treatment.

detected for standing stock N ($P \geq 0.46$). There was a significant increase in standing stock N over time for stunted *S. alterniflora* ($P = 0.02$; Fig. 4).

Plant community structure.—Community structure remained unchanged throughout the experiment with no community shifts detected between treated and reference creeks ($P > 0.05$; Fig. 5). We found no evidence of a landward encroachment of tall *S. alterniflora* with nutrient enrichment as there was no difference in its percent cover between treatments (Fig. 6). Significant differences occurred for frequency of occurrence of species for the third and eighth year of enrichment only (Appendix S1: Table S8). In both enriched and reference creeks, *D. spicata* increased in cover over time (data not shown).

DISCUSSION

Overall, we found mild bottom-up stimulation of salt-marsh plants after nine years of nutrient enrichment, with the strongest effects in the low marsh and weakest effects in the high marsh. Nutrient responses at the shoot level were generally small, resulting in no differences in end-of-season-live biomass. Contrary to the hypothesis of a nutrient-driven increase in *S. alterniflora* and *D. spicata* dominance (Levine et al. 1998), plant community structure remained unchanged. We submit that the relatively modest effects of nutrient addition compared to past studies reflects three unique aspects of our study: (1) the use of loading rates that are more typical of anthropogenic eutrophication, (2) indirect fertilization of the wetland by addition of nutrients to the incoming tidal waters rather than direct application of fertilizer, and (3) the use of nitrate fertilizer, the form of nitrogen typically supplied to eutrophic wetlands, rather than ammonium fertilizer.

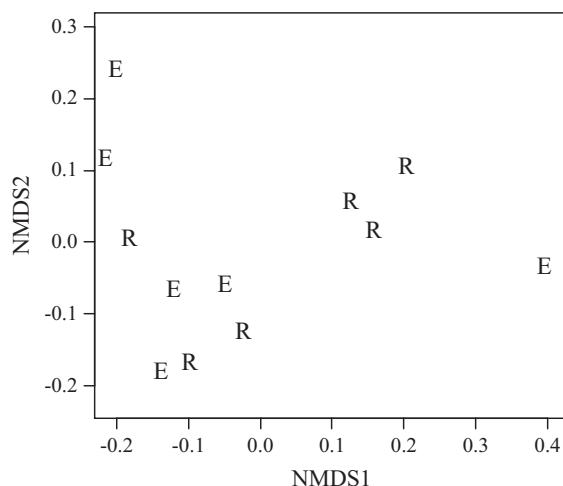


FIG. 5. Non-metric multi-dimensional scaling plot of plant communities (measured as percent cover) in year 8 of enrichment. Based on analysis of similarities, there are no differences between the two communities ($P = 0.32$). Abbreviations are E, enriched communities; R, reference communities.

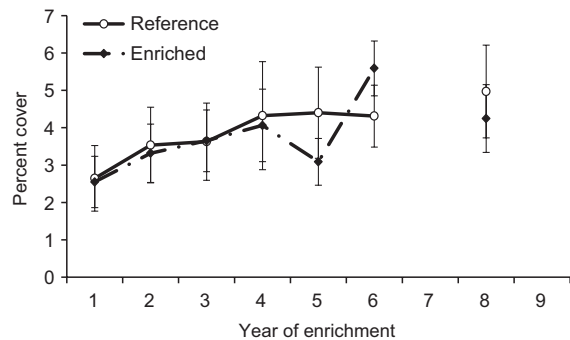


FIG. 6. Mean ($1 \pm SE$) percent cover of tall *Spartina alterniflora* along vegetation transects along enriched (dashed line) and reference (solid line) creeks. No data available for year 7. No significant effect of year or enrichment.

Our more realistic loading rates in the high marsh are at least 10 times lower than many plot-level studies ($19 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ this study vs. $105\text{--}225 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$; Levine et al. 1998, Darby and Turner 2008, Fox et al. 2012, Morris et al. 2013). The high levels used in previous studies were meant to ensure a response so as to study processes and mechanisms associated with enrichment, but in so doing may also have stimulated changes that are not necessarily seen under more realistic levels of enrichment. For instance, studies that have reported a change in *S. alterniflora* dominance with nutrient enrichment perturbed the system with an order of magnitude higher levels of N to elucidate interspecific interactions that drive local zonation patterns (Levine et al. 1998, Emery et al. 2001, Pennings et al. 2005). Our nine year dataset under more realistic scenarios clearly indicates that moderate levels of nutrient enrichment applied via the water column at realistic concentrations is not sufficient to alter competitive dynamics among plant species.

The positive shoot-level response of tall *Spartina alterniflora* may result from the reallocation of resources from below- to aboveground tissues with nutrient enrichment. In our experiment, nutrients decreased belowground biomass (roots and rhizomes) of tall *S. alterniflora* by 20% (Deegan et al. 2012), which is a common response of saltmarsh plants to nutrient enrichment (Darby and Turner 2008, Graham and Mendelsohn 2014, Valiela 2015). An alternative hypothesis is that tall *S. alterniflora* in our sites are nutrient limited. However, tall *S. alterniflora* occupies a lower elevation in the tidal frame and is therefore regularly flooded (twice daily in the current study system). Regular flooding enhances plant growth through regular nutrient delivery and flushing of the soils. As a result, tall *S. alterniflora* is not believed to be nutrient limited (Morris et al. 2013). This conclusion is consistent with the lack of N-content response of the leaves to enrichment in our study. While shoot height and mass increased, it did not translate into increased

aboveground biomass due to declines in stem density with enrichment.

Unlike low marsh plants, high-marsh plants are typically nutrient limited and frequently respond strongly to nutrient enrichment (Mendelsohn 1979, Levine et al. 1998, Bertness and Pennings 2000, Emery et al. 2001, Morris et al. 2013). High-marsh plants in our study, however, responded only weakly to nutrient enrichment. Stunted *S. alterniflora* was the only high-marsh plant that consistently responded to nutrient enrichment and only for shoot-specific mass and foliar N. Plant communities remained stable throughout our experiment, contrary to previous studies that have suggested nutrients can drive changes in saltmarsh plant community structure (Levine et al. 1998, Bertness and Pennings 2000, Emery et al. 2001, Wigand et al. 2004).

The high marsh may have been unresponsive to our nutrient enrichment because the level of nutrient exposure was insufficient to elicit strong responses. Because nitrogen was added to tidal flood waters, hydroperiod controlled loading rates to different parts of the marsh. The low marsh and high marsh differ in the length of inundation, which results in hydrologically driven loading rates of $171 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ in the low marsh and $19 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ in the high marsh. As a result, the strongest plant responses in our study system were in the low marsh (this study, Deegan et al. 2012). Similarly, stronger responses of benthic invertebrates were found in the low marsh relative to the high marsh (Fleeger et al. 2008). Thus, given tidal delivery of nutrients in estuarine water, marsh responses to eutrophication are likely to shift along a hydrologically driven loading gradient.

The supply of nitrate via flood waters can also influence nutrient retention and availability to plants (Mendelsohn 1979). Marshes can retain up to 95% of supplied N from dry fertilizer broadcast on the marsh surface or buried in the rooting zone as slow-release granules (Brin et al. 2010). Based on estimates of net nitrate retention and nitrate concentrations measured in ebbing tidal water in the current study, ~50% of the nitrate added to the enriched creeks is exported on the following ebb tide (Drake et al. 2009). The reduced N retention in our study is due, in part, to limited nutrient uptake related to the short flooding time of the high marsh. Despite its considerable area (~80% of the total marsh surface), the high marsh is inundated only 4% of the time, which restricts contact time for plant uptake.

The form of N can also determine plant responses to N enrichment (Mendelsohn 1979). The majority of studies that have shaped salt marsh theory have primarily used either ammonium- or urea-N, which itself is quickly converted to ammonium (Valiela and Teal 1974, Levine et al. 1998, Langley and Magonigal 2010, Fox et al. 2012). In contrast, we used nitrate-N in our experiment to mimic the most common form of anthropogenic N in estuarine surface waters (Cloern 2001). Early studies of *S. alterniflora* have clearly demonstrated that ammonium enrichment promotes higher growth

over nitrate (Mendelsohn 1979), particularly with high salinities (Hessini et al. 2013).

Four factors may explain the modest aboveground plant responses to nitrate-N compared to studies that used ammonium-N. First, nitrate assimilation has higher energetic costs (Lambers et al. 1998) and lower root nitrate uptake kinetics when compared to ammonium (Mendelsohn and Morris 2000). Based on recent data (2015), nutrient enrichment elevated porewater nitrate threefold in marsh sediments; however, porewater nitrate was still an order of magnitude lower than porewater ammonium, which did not vary significantly between treatments (T. Mozdzer, *unpublished data*). Second, foliar uptake rates of ammonium are six times higher than nitrate in *S. alterniflora* even given an order of magnitude difference in N-availability for nitrate in the water column (Mozdzer et al. 2011). Third, as an anion, nitrate-N moves freely through our system and is likely exported before it can be used. In contrast, ammonium, a cation, readily binds to soil particles ensuring availability to plants in the short- and long-term (Brin et al. 2010). Fourth, although porewater nitrate was elevated, increased denitrification may have limited N-availability to plants on the high marsh platform. Koop-Jakobsen and Giblin (2010) found a 30% increase in denitrification rate with nutrient enrichment in the high marsh in the current study system. Assuming that the microbes out-compete the plants, higher denitrification rates would further reduce the NO_3^- available to plants on the high marsh. Combined with low loading rates due to infrequent inundation, competitive losses via denitrification reduced exposure of the high marsh to nitrate enrichment.

The form of nitrogen delivered to an ecosystem may have implications for other types of ecosystems as well. For instance, species-specific preference for either ammonium vs. nitrate uptake is common in plants (Falkengren-Grerup 1995). In terrestrial grassland ecosystems, N enrichment is due predominately to atmospheric deposition, which is not uniform in its relative abundance of N forms (Zhang et al. 2012). Thus, differential responses of ecosystems to similar N-deposition rates may be driven, in part, by the relative abundance of different forms of N and plant species preferences. Given that many N-response studies, in both terrestrial and marine ecosystems, use fertilizers with both ammonium and nitrate (Levine et al. 1998, Silliman and Bertness 2002, Hautier et al. 2009, Isbell et al. 2013), the relative contributions of these two N ions in driving observed changes is unclear.

Although our analysis focused on aboveground plant community responses to nutrient enrichment, our results do not imply an overall weak response by the ecosystem to nutrient enrichment. The most striking response is the nutrient-induced destabilization of the creek-bank habitat (the zone of tall *S. alterniflora*), leading to marsh loss as a result of decreased belowground production and increased microbial respiration of organic matter (Deegan et al. 2012). Additionally, we have seen strong

bottom-up stimulation of the food web, including invertebrates (Johnson et al. 2009, Johnson 2011, Johnson and Short 2013) and nekton (Deegan et al. 2007, Lockfield et al. 2013), most likely related to increases in benthic algae (Pascal and Fleeger 2013, Pascal et al. 2013).

Our work has implications for conceptual and theoretical models of how eutrophication may impact salt-marsh ecosystems that are predicated on the response of foundation species (e.g., salt marsh plants). For instance, ecogeomorphic models of salt marshes predict that increases in aboveground plant biomass will lead to increased sediment accretion as a result of enhanced sediment trapping by stems (Morris et al. 2002). This sediment accretion, in turn, may help salt marshes maintain elevation with sea-level rise (Kirwan and Megonigal 2013). Thus, one might predict a positive effect of nutrients on marsh accretion due to nutrient-enhanced aboveground biomass (Morris et al. 2002, Graham and Mendelsohn 2014). While this may bear out at unrealistically high levels of N loading in higher elevations, under realistic eutrophic conditions we demonstrate that loading rates may not be sufficient to enhance aboveground biomass and in turn have no significant effect on sediment accretion (LeMay 2007). Furthermore, eutrophication may weaken salt-marsh stability due to loss of soil organic matter (Darby and Turner 2008, Deegan et al. 2012).

CONCLUSIONS

Eutrophication is a global threat to wetlands. Our results demonstrate that under real-world conditions of wetland eutrophication, salt marsh foundation species may not follow the results of previous plot-level studies. The relatively modest increases at the shoot level followed the predictions of bottom-up theory to nutrient enrichment in the low marsh, but we did not observe any significant changes in the high marsh. From an applied perspective, our results demonstrate that with tidal delivery, realistic water column concentrations of nitrate may not achieve N-loading rates high enough to elicit ecologically significant responses on the high marsh, at either the individual species or plant community level. Clearly, our results suggest a higher N watershed loading rate/threshold or alternate form of N is needed to induce responses found in plot level studies. From an ecological perspective, while nutrient enrichment may affect biodiversity and ecosystem functioning at high nutrient loads (Levine et al. 1998, Isbell et al. 2013), our results highlight the importance of considering factors that may limit nutrient loading and in turn limit responses. More broadly, the magnitude of biological response to bottom-up forcing may be driven, in part, by the magnitude and form of the resource supplied. Given that nutrient enrichment is pervasive in a range of ecosystems, from lakes to grasslands to estuaries, our study underscores the importance of the delivery mechanisms and relative abundances of N forms as important factors that may affect loading rates, and thus species, community, and ecosystem responses.

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LITERATURE CITED

- Bertness, M. D. 1991. Zonation of *Spartina* spp. in New England salt marshes. *Ecology* 72:138–148.
- Bertness, M. D., P. Ewanchuk, and B. R. Silliman. 2002. Anthropogenic modification of New England salt marsh landscapes. *Proceedings of the National Academy of Science USA* 99:1395–1398.
- Bertness, M. D., and S. C. Pennings. 2000. Spatial variation in process and pattern in salt marsh plant communities. Pages 39–58 in M. Weinstein, editor. *Concepts and controversies in tidal marsh ecology*. Kluwer Press, Boston, Massachusetts, USA.
- Brin, L. D., I. Valiela, D. Goehring, and B. Howes. 2010. Nitrogen interception and export by experimental salt marsh plots exposed to chronic nutrient addition. *Marine Ecology Progress Series* 400:3–17.
- Chaisson, C. 2012. Factors influencing stem density of creek-bank *Spartina alterniflora* in a New England salt marsh. Thesis. Connecticut College, New London, Connecticut, U.S.
- Chapin, F. S., P. M. Vitousek, and K. Van Cleave. 1986. The nature of nutrient limitation in plant communities. *American Naturalist* 127:48–58.
- Cloern, J. E. 2001. Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology Progress Series* 210:223–253.
- Crain, C. M. 2007. Shifting nutrient limitation and eutrophication effects in marsh vegetation across estuarine salinity gradients. *Estuaries and Coasts* 30:26–34.
- Darby, F. A., and R. E. Turner. 2008. Effects of eutrophication on salt marsh root and rhizome biomass accumulation. *Marine Ecology Progress Series* 363:63–70.
- Deegan, L. A., et al. 2007. Susceptibility of salt marshes to nutrient enrichment and predator removal. *Ecological Applications* 17:S42–S63.
- Deegan, L. A., D. S. Johnson, R. S. Warren, B. J. Peterson, J. W. Fleeger, S. Fagherazzi, and W. M. Wollheim. 2012. Coastal eutrophication as a driver of salt marsh loss. *Nature* 490:388–392.
- Drake, D. C., B. J. Peterson, K. A. Galvan, L. A. Deegan, C. Hopkinson, J. M. Johnson, K. Koop-Jakobsen, L. E. Lemay, and C. Picard. 2009. Salt marsh ecosystem biogeochemical responses to nutrient enrichment: a paired ^{15}N tracer study. *Ecology* 90:2535–2546.
- Emery, N., P. Ewanchuk, and M. D. Bertness. 2001. Nutrients, mechanisms of competition and the zonation of plants across salt marsh landscapes. *Ecology* 82:2471–2485.
- Falkengren-Grerup, U. 1995. Interspecies differences in the preference of ammonium and nitrate in vascular plants. *Oecologia* 102:305–311.

- Fleece, J. W., D. S. Johnson, K. A. Galván, and L. A. Deegan. 2008. Top-down and bottom-up control of infauna varies across the saltmarsh landscape. *Journal of Experimental Marine Biology and Ecology* 357:20–34.
- Fox, L., I. Valiela, and E. L. Kinney. 2012. Vegetation cover and elevation in long-term experimental nutrient-enrichment plots in Great Sippewissett salt marsh, Cape Cod, Massachusetts: implications for eutrophication and sea-level rise. *Estuaries and Coasts* 35:445–458.
- Galloway, J. N., A. R. Townsend, J. W. Erisman, M. Bekunda, Z. Cai, J. R. Freney, L. A. Martinelli, S. P. Seitzinger, and M. A. Sutton. 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320:889–892.
- Graham, S. A., and I. A. Mendelsohn. 2014. Coastal wetland stability maintained through counterbalancing accretionary responses to chronic nutrient enrichment. *Ecology* 95: 3271–3283.
- Hautier, Y., P. A. Niklaus, and A. Hector. 2009. Competition for light causes plant biodiversity loss after eutrophication. *Science* 324:636–638.
- He, Q., and B. R. Silliman. 2015. Biogeographic consequences of nutrient enrichment for plant–herbivore interactions in coastal wetlands. *Ecology Letters* 18:462–471.
- Hessini, K., K. B. Hamed, M. Gandour, M. Mejri, C. Abdelly, and C. Cruz. 2013. Ammonium nutrition in the halophyte *Spartina alterniflora* under salt stress: Evidence for a priming effect of ammonium? *Plant and Soil* 370:163–173.
- Isbell, F., D. Tilman, S. Polasky, S. Binder, and P. Hawthorne. 2013. Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity. *Proceedings of the National Academy of Science USA* 110:11911–11916.
- Johnson, D. S. 2011. High marsh invertebrates are susceptible to eutrophication. *Marine Ecology Progress Series* 438: 142–152.
- Johnson, D. S., J. W. Fleece, and L. A. Deegan. 2009. Large-scale manipulations reveal top-down and bottom-up controls interact to alter habitat utilization by saltmarsh fauna. *Marine Ecology Progress Series* 377:33–41.
- Johnson, D. S., J. W. Fleece, K. A. Galván, and E. B. Moser. 2007. Worm holes and their space-time continuum: spatial and temporal variability of macroinfaunal annelids in a Northern New England salt marsh. *Estuaries and Coasts* 30:226–237.
- Johnson, D. S., and B. J. Jessen. 2008. Do spur-throated grasshoppers, *Melanoplus* spp. (Orthoptera: Acrididae), exert top-down control on smooth cordgrass *Spartina alterniflora* in northern New England? *Estuaries and Coasts* 31:912–919.
- Johnson, D. S., and M. I. Short. 2013. Chronic nutrient enrichment increases the density and biomass of the mudsnail *Nassarius obsoletus*. *Estuaries and Coasts* 36:28–35.
- Kirwan, M. L., and J. P. Megonigal. 2013. Tidal wetland stability in the face of human impacts and sea-level rise. *Nature* 504:53–60.
- Koop-Jakobsen, K., and A. E. Giblin. 2010. The effect of increased nitrate loading on nitrate reduction via denitrification and DNRA in salt marsh sediments. *Limnology and Oceanography* 55:789–802.
- Lambers, H., F. S. Chapin, and T. L. Pons. 1998. *Plant physiological ecology*. Springer, Berlin, Germany.
- Langley, J. A., and J. P. Megonigal. 2010. Ecosystem response to elevated CO₂ levels limited by nitrogen-induced plant species shift. *Nature* 466:96–99.
- LeMay, L. E. 2007. The impact of drainage ditches on salt marsh flow patterns, sedimentation and morphology: Rowley River, Massachusetts. Thesis. School of Marine Science, College of William and Mary, Gloucester Point, Virginia, USA.
- Levine, J., S. J. Brewer, and M. D. Bertness. 1998. Nutrient availability and the zonation of marsh plant communities. *Journal of Ecology* 86:285–292.
- Lockfield, K., J. W. Fleece, and L. A. Deegan. 2013. Mummichog *Fundulus heteroclitus* responses to long-term, whole-ecosystem nutrient enrichment. *Marine Ecology Progress Series* 492:211–222.
- Mendelsohn, I. A. 1979. Nitrogen metabolism in the height forms of *Spartina alterniflora* in North Carolina. *Ecology* 60:574–584.
- Mendelsohn, I. A., and J. T. Morris. 2000. Ecophysiological controls on the growth of *Spartina alterniflora*. Pages 59–80 in N. P. Weinstein and D. A. Kreeger, editors. *Concepts and controversies in tidal marsh ecology*. Kluwer, Dordrecht, the Netherlands.
- Miller, W. R., and F. E. Egler. 1950. Vegetation of the Wequetequoick-Pawcatuck tidal marshes, Connecticut. *Ecological Monographs* 30:143–172.
- Morris, J. T., P. V. Sundareswar, C. T. Nietch, B. Kjerfve, and D. R. Cahoon. 2002. Responses of coastal wetlands to rising sea level. *Ecology* 83:2869–2877.
- Morris, J. T., K. Sundberg, and C. S. Hopkinson. 2013. Salt marsh primary production and its responses to relative sea level and nutrients. *Oceanography* 26:74–80.
- Mozdzer, T. J., M. Kirwan, K. J. McGlathery, and J. C. Zieman. 2011. Nitrogen uptake by the shoots of smooth cordgrass *Spartina alterniflora*. *Marine Ecology Progress Series* 433:43–52.
- Niering, W. A., and R. S. Warren. 1980. Vegetation patterns and processes in New England salt marshes. *BioScience* 30:301–307.
- Nixon, S. W., and C. A. Oviatt. 1973. Ecology of a New England salt marsh. *Ecological Monographs* 43:463–498.
- Pardo, L. H., et al. 2011. Effects of nitrogen deposition and empirical nitrogen critical loads for ecoregions of the United States. *Ecological Applications* 21:3049–3082.
- Parsons, T. R., Y. Maita, and C. M. Lalli. 1984. *A manual of chemical and biological methods for seawater analysis*. Pergamon Press, Elmsford, New York.
- Pascal, P.-Y., and J. W. Fleece. 2013. Diverse dietary responses by saltmarsh consumers to chronic nutrient enrichment. *Estuaries and Coasts* 36:1115–1124.
- Pascal, P.-Y., J. W. Fleece, H. T. S. Boschker, H. M. Mitwally, and D. S. Johnson. 2013. Response of benthic food web to short-term and long-term nutrient enrichment in saltmarsh mudflats. *Marine Ecology Progress Series* 474: 27–41.
- Pennings, S. C., M. Bestor-Grant, and M. D. Bertness. 2005. Plant zonation in low-latitude salt marshes: disentangling the roles of flooding, salinity and competition. *Journal of Ecology* 93:159–167.
- R Development Team. 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Silliman, B. R., and M. Bertness. 2002. A trophic cascade regulates salt marsh primary production. *Proceedings of the National Academy of Science USA* 99:10500–10505.
- Smith, S. M., and S. Warren. 2007. Determining ground surface topography in tidal marshes using watermarks. *Journal of Coastal Research* 23:265–269.
- Stevens, C. J., N. B. Dise, J. O. Mountford, and D. J. Gowing. 2004. Impact of nitrogen deposition on the species richness of grasslands. *Science* 303:1876–1879.
- USEPA (U.S. Environmental Protection Agency). 2005. National Coastal Condition Report. U.S. Environmental Protection Agency, Washington, D.C., USA. <http://www.epa.gov/owow/oceans/nccr/2005/>

- Valiela, I. 1984. Marine ecological processes. Springer-Verlag, New York, New York, USA.
- Valiela, I. 2015. The Great Sippewissett Salt Marsh plots: some history, highlights, and contrails from a long-term study. *Estuaries and Coasts* 38:1099–1120.
- Valiela, I., et al. 1992. Couplings of watersheds and coastal waters: sources and consequences of nutrient enrichment in Waquoit Bay, Massachusetts. *Estuaries* 15:443–457.
- Valiela, I., and J. M. Teal. 1974. Nutrient limitation in salt marsh vegetation. Pages 547–563 in R. J. Reimold and W. H. Queen, editors. *Ecology of halophytes*. Academic Press, New York, New York, USA.
- Vieillard, A. and R. W. Fulweiler. 2012. Impacts of Long-term Fertilization on Salt Marsh Tidal Creek Benthic Nutrient and N₂ Gas Fluxes. *Marine Ecological Progress Series* 471: 11–22.
- Vitousek, P. M., R. W. Howarth, G. E. Likens, P. A. Matson, D. Schindler, W. H. Schlesinger, and G. D. Tilman. 1997. Human alteration of the global nitrogen cycle: causes and consequences. *Issues in Ecology* 1:1–17.
- Wigand, C., G. B. Thursby, R. A. McKinney, and A. F. Santos. 2004. Response of *Spartina patens* to dissolved inorganic nutrient additions in the field. *Journal of Coastal Research* 45:134–149.
- Wood, E. D., F. A. Armstrong, and F. A. Richards. 1967. Determination of nitrate in sea water by cadmium-copper reduction to nitrite. *Journal of the Marine Biological Association of the United Kingdom* 47:23–31.
- Zhang, L., D. J. Jacob, E. M. Knipping, N. Kumar, J. W. Munger, C. C. Carouge, A. van Donkelaar, Y. X. Wang, and D. Chen. 2012. Nitrogen deposition to the United States: distribution, sources, and processes. *Atmospheric Chemistry and Physics* 12:4539–4554.

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/eap.1402/supinfo>

DATA AVAILABILITY

Data associated with this paper have been deposited in the Plum Island Estuary Long Term Ecological Research data archive.

Plant shoot density data: <http://ecosystems.mbl.edu/PIE/data/LTE/LTE-TIDE-MarshPlantStemCount.html>

Plant shoot traits data: <http://ecosystems.mbl.edu/PIE/data/LTE/LTE-TIDE-MarshPlantShootStats.html>

Plant species frequency data: <http://ecosystems.mbl.edu/PIE/data/LTE/LTE-TIDE-MarshPlantFreq.html>

Plant species percent cover data: <http://ecosystems.mbl.edu/PIE/data/LTE/LTE-TIDE-MarshPlantCover.html>

Foliar carbon and nitrogen content data: <http://ecosystems.mbl.edu/PIE/data/LTE/LTE-TIDE-MarshPlantCHN.html>